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Fourth Annual Report
March 1, 2002 – February 28, 2003

A Sustainable IPM Program for the African Armyworm, *Spodoptera exempta*

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SECTION I

Executive Summary

Our field research for the reporting period was super-imposed on an existing project administered by Dr. Z.R. Khan (ICIPE, Mbita Point Field Station), evaluating cultural control strategies for cereal stem borer and the weed, *Striga*, both of which are persistent limiting factors to maize production in East Africa. Armyworm are less frequent pests, but are unaffected by these management tactics; thus, in an outbreak year, crops can be totally devastated by armyworm feeding. It is desirable to bring together all relevant pest control components in a truly integrated pest management strategy, and our collaboration enables more diverse crop protection measures to ultimately be implemented.

We assessed effects of intercropping maize with *Desmodium* (which suppresses *Striga* and repels stem borers) on soil microarthropod biodiversity. Biodiversity is essential to sustainable agricultural systems. Abundant populations of soil Collembola, for example, are associated with healthy and productive soils. Their key role is soil decomposition and nutrient recycling is well documented. Our goal was to determine the impact different crop treatments on soil microarthropods, and by so doing, attempt to 'measure' additional benefits derived from adopting these production practices.

In field trials conducted at ICIPE's Mbita Point Field Station and in farmer's fields in the nearby Lambwe Valley, we consistently observed higher populations of Collembola and beneficial soil mites in plots/fields where maize was intercropped with *Desmodium* vs. those where a maize monocrop was planted. Use of straw mulch also benefited these soil microarthropods. We speculate that a combination of moisture conservation due to the ground cover provided by this vegetation, and the greater availability of organic material for these organisms to feed on, contributed significantly to their greater abundance. Higher populations will result in increased soil fertility, and improved soil structure over time. We believe it would be of great benefit, and a subject worthy of future investigation, to encourage farmers to use intercrops and/or to promote some sort mulching to enhance soil conditions.

We are also able to report the identity of several species of microarthropods recovered during earlier phases of this project. Two publications have already been produced describing species new to science. While the identity of many specimens remains to be determined, we have solicited the cooperation of experts in taxonomy from Europe, and will be able to provide a complete listing of these organisms in future publications. The microarthropod fauna of East Africa is poorly described, so this represents a significant contribution to the collective body of scientific knowledge generated by this project.

Finally, in a series of laboratory trials carried out by the US partner, effects of the control products tested in this project – *Bacillus thuringiensis* var. *aizawai* (Bt) and neem seed extract – on Collembola was evaluated. Bt had no negative effects at all test concentrations used. Neem seed extract – administered orally or mixed in soil – had a direct impact on collembolan longevity, egg production, and population growth. While such effects cannot be directly extrapolated to predict what would happen under field conditions (for example, in the trials,

Collembola were constantly fed on contaminated diet, and it is unlikely that such high concentrations would reach Collembola naturally in their food, or in the soil), they provide us with an indication of potential risks that now need to be evaluated under conditions more closely resembling those experienced in the field.

Bt seems to be a completely safe and viable control agent, and although use of neem may not be entirely without risk, it is a much safer and more acceptable choice than the insecticides currently used against AAW in East Africa. Both Bt and neem have been shown to be highly effective against this pest. Ultimately, our findings must be used to guide decisions on selection and use of pest management tools for AAW in light of the relative risks posed by these materials compared to those posed by toxic synthetic pesticides, and the benefits, in terms of increased food production, that would be gained from their use. Furthermore, neem seed extract can be indigenously produced in the areas where it is needed at extremely low cost, and in a timely and responsive fashion. Thus, the rural farming community could have ready access to this control agent. Bt can be produced in-country and neem trees thrive in parts of Kenya. Both may be considered as inexpensive and renewable pest management resources.

Research Goal

- To incorporate a Bt preparation and biorational products into a sustainable IPM program for the African armyworm.

Research Objectives

1. Evaluate effects of intercropped vs. mono-cropped maize on microarthropod biodiversity
2. Describe and document microarthropods recovered from farm soils collected in the Lambwe Valley region of Kenya
3. Assess potential non-target effects of Bt and neem in laboratory trials against Collembola

SECTION II

Collaboration, Travel and Managerial Issues

1. The project was initiated in March 1998 when the main outbreak season of AAW was already ongoing. Thus, it was our intention to spread the study over four consecutive years, ending March 1, 2002. However, due to health problems of the Principal Investigator, we requested and received an extension to February 28, 2003. The following Fourth Annual Report covers activities undertaken during this period.

2. The report summarizes experimental findings on non-target organisms carried out in the USA, and an analysis of microarthropod samples collected in maize fields in Kenya during the 2000 outbreak season.

3. Twice, (Fall 2002, Jan- Feb 2003), the Israeli team arranged to make a scientific trip to Kenya, to work together with Kenyan collaborators, to complete non-target collections and training, and educational commitments to graduate students working on this project and the local farming community. However, because of local riots prior to and during the presidential election in Kenya, the first trip was cancelled. The second trip also had to be cancelled following the terrorist attack against Israeli tourists in Mombasa.

4. Several publications were completed during the current reporting period. As part of the 'biodiversity component' of the project, a new species of Protura was described (Szeptycky & Broza, In Press; see Appendix), and a new species of Pauropoda was described by Scheller (Insect Science and its Application 19, 1999). Finally, in October 2002 Ms. Ayelet Shavit graduated from the University of Tel Aviv. Her M.Sc. thesis (in Hebrew) incorporates many of the findings included in the Third Annual Report. Additional manuscripts are currently in preparation.

5. The Final Report will be completed and submitted by early October 2003.

SECTION III

NON TARGET SOIL MICROARTHROPODS AS BIO-INDICATORS IN INTERCROPPED MAIZE FIELDS IN SUBA DISTRICT, WESTERN KENYA

This research was carried out in cooperation with Dr. Zeyaur Rahman Khan, ICIPE, Mbita Point Field Station, Suba District, Kenya.

3.1 Introduction and Justification

The Push-Pull System

The 'push-pull' strategy involves trapping stemborers on highly susceptible trap plants (pull) and driving them away from the maize crop using repellent intercrops (push). Plants, which repel stemborers as well as inhibit striga have also been identified. On-farm trials with more than 600 farmers in Kenya have confirmed that these approaches, conducted separately and together, work and result in significant yield increases. In 2001 the system was used by 1100 small-scale farmers. This pest management approach has been developed by ICIPE researchers in collaboration with the Kenya Agricultural Research Institute (KARI), the Kenya Ministry of Agriculture, and the Institute of Arable Crops Research, Rothamsted, UK (Khan et al, 1997a, 1997b, 2000).

The plants which are used as trap or repellent plants in a push-pull strategy are Napier grass *(Pennisetum purpureum),* Sudan grass *(Sorghum vulgare sudanense)*, molasses grass *(Melinis minutiflora)* and silver leaf desmodium *(Desmodium uncinatum)*. Napier grass and Sudan grass have shown potential for use as trap plants, whereas molasses grass and silverleaf desmodium repel ovipositing stemborers. Molasses grass, when intercropped with maize, not only reduced infestation of the maize by stemborers, but also increased stemborer parasitism by a natural enemy, *Cotesia sesamiae*. In addition, *Desmodium,* when intercropped with maize, inhibited striga. All four plants are of economic importance to farmers in eastern Africa as livestock fodder, and have shown great potential in stemborer and striga management in farmer participatory trials. These innovations were found to be appropriate and acceptable to these small-scale farmers in Kenya.

Justification

The main objective of the project was to strengthen sustainable agricultural practices in general for crop production in Western Kenya, and specifically for management of the African armyworm [AAW] in maize. During trips to field sites in the Suba district during the rainy season, we observed widespread parasitism of maize plants by *Striga* spp. The intercropping system developed to eliminate this weed and to protect against stem borers could be incorporated into an improved, sustainable IPM program for AAW resulting from our studies. Combining this intercropping approach into an IPM program for AAW is a highly appropriate application of this technology that would enhance sustainable agriculture in this region and beyond.

Soil Microarthropods as Bioindicators and Decomposers

Bio-indicators. This aspect is dealt with extensively in another chapter of this report (see Section II).

Microarthropods as decomposers and their impact on soil fertility. Extensive literature (e.g., Peterson and Luxton 1983, Broza et al. 1999) has summarized the ecological significance of soil microarthropods in the decomposition process, first of all by diminution of litter and other dead organic components. They also increase the porosity [and thus aeration] of the soil and, by typically being mold feeders, play an important role in the bacterial/fungal balance in the soil.

In this study, we extracted soil arthropods from standardized samples using modified Berlese funnels. By comparing the number and diversity of the arthropods recovered, we intended to derive information on the relative potential of the soils for crop production, assuming that higher values of these parameters indicate greater organic richness and soil quality, and hence greater suitability for sustainable farming. Such data may serve as indicators only, and represent a preleminary stage of the research. The next stages – which are beyond the mandate of the current research project - may include various methods to measure the net value of nutrients in the soil (e.g., C:N balance, P^{++}), higher crop yields etc., in the presence/absence of the faunal components screened in this study.

3.2 Material & Methods

Study sites

1. In Mbita Point Field Station (MPFS)

Field 3A: Two field plots, equivalent in size to those commonly found in local farmer's fields, were planted on 3/4/00; one plot was planted with maize intercropped with *Desmodium*, and the second with a maize mono-culture. Both plots were irrigated until 15/6/00, and sampled on 30/6/00.

Field 2D: Experimental plots (approx. 10 x 6m) arranged in a Latin-square design incorporating the following replicated treatments: Maize-monoculture; Maize + *Desmodium* [Des]; Maize + Straw [Str]; Maize + Nitrogen [N]; Maize + Des + N; Maize + Str + N (4 plots/treatment).

2. Local Farms

Field plots were situated on three farms near Sindo and other villages in the Lambwe-Valley region, Suba District. Sample fields were planted with a maize-*Desmodium* intercrop, or a maize-only monocrop.

Sampling methods, extraction, examination, and identification

Plots in field 3A at MPFS were sampled using a standard soil corer to a depth of 4 cm, and included both soil and litter layers. Six samples were collected at random throughout each treatment plot. In Field 2D only four soil samples were taken in each replicate plot of the following three treatments: maize + *Desmodium*, maize + straw, and maize monocrop. Litter samples were collected in 2D using a small hand trowel. In all cases, samples were extracted using Berlese funnels and microarthropods preserved in 95% ethanol. In the farmer's fields, soil and litter samples were collected as described above. Six samples were taken at random in each field, on each farm (total 18 soil/litter samples per field treatment).

3.3 Results & Discussion

Microarthropods extracted from soil samples are shown in Fig.1 Arthropods extracted from samples taken in the Desmodium-Maize intercropping plot (black columns) are presented together with those from the mono-culture maize plots (white columns). Soil microarthropods were sorted and identified into 19 different taxa; the mean of six samples from each treatment block was calculated. The five major groups of microarthropods recovered are shown in Fig. 1. They include the order Acari (1), the three main families [found] of Collembola (2-4) and Myriapoda (5).

There are striking differences in the number of arthropods recovered from the intercrop vs. mono-maize field in all taxa.

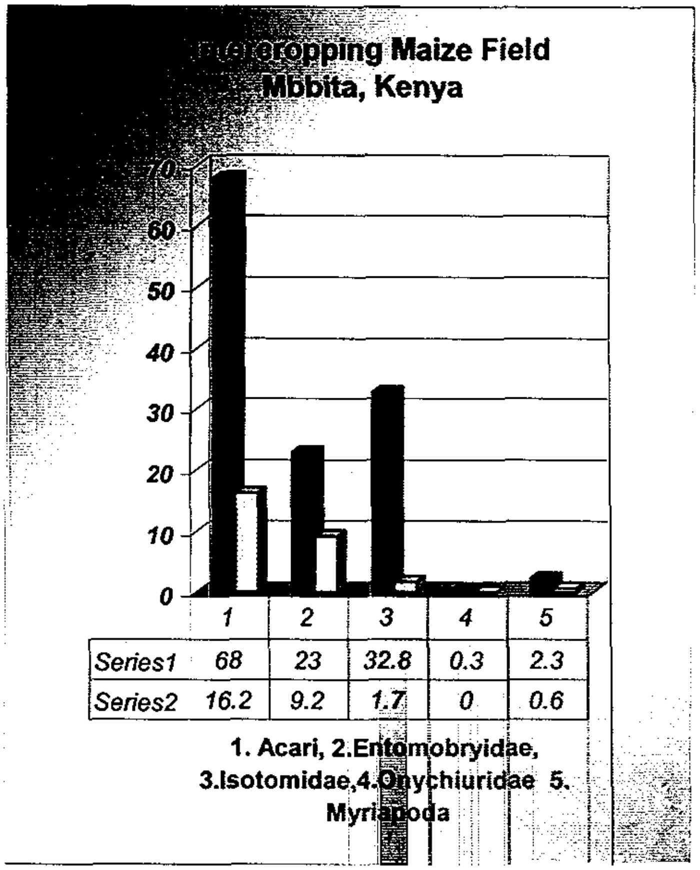
Myriapoda & Acari

All four classes of Myriapoda (Pauropoda- to- Symphyla) were clustered together in this figure (as # 5) and onward. The Myriapods include the largest soil arthropods in the soil. Thus while relatively few were recovered, they comprise a greater proportion of the total biomass, and the mechanical effects of their activity in the soil is higher than can be appreciated from the current numerical summary. Acari form a diverse group and further taxonomic division of this group is needed for comparative analysis between different treatment plots. Groups 1 and 5 may both include predators, but most are decomposers and fungal feeders.

Collembola

Representatives of two families [and super-families] were dominant in maize: the Entomobroyidea, which are epigeic and largely active in the litter layers; and the Isotomidae, which roam just below and within the humus layer. Three other families were either rare – Sminthuroidea and Onychiuridae - or absent – Hypogastruridae - in these samples, although they were found in the natural habitats (see below, Fig 7b).

The treatments applied to field 2D were made to determine whether *Desmodium* protects maize and inhibits the growth of Striga by mechanical means or by other biological interactions. Our soil sampling was super-imposed on these treatment plots to detect differences in soil microarthropod populations according to the treatment applied, i.e., maize + *Desmodium* [blue], maize + soil covered with straw [purple], and mono-culture maize [yellow] (Fig. 2, 3). In all treatments (including those in Figures 3-4 below) and all taxonomic groups (apart from the Acari in Fig. 2), microarthropod populations were much higher in the intercropped plots. The simulated litter cover (maize straw) created conditions that favored the development of significantly higher numbers of soil microarthropods. The straw may have provided some mechanical protection, as well as an unlimited source of food, i.e., organic matter. However, the



	1	2	3	4	5
Series1	68	23	32.8	0.3	2.3
Series2	16.2	9.2	1.7	0	0.6

Fig. 1. The mean numbers of soil microarthropods sorted into five taxa [1-5], as sampled in Maize/Desmodium intercropped field [Black column, Series 1], in contrast to monoculture-maize field [white column, Series 2], (June 15, 2000, field 3A, MPFS).

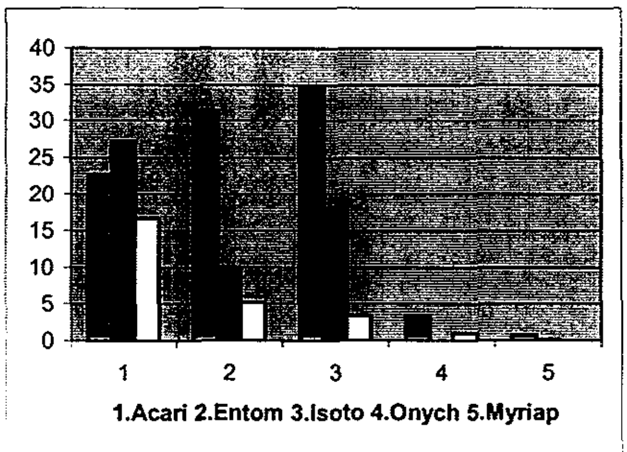
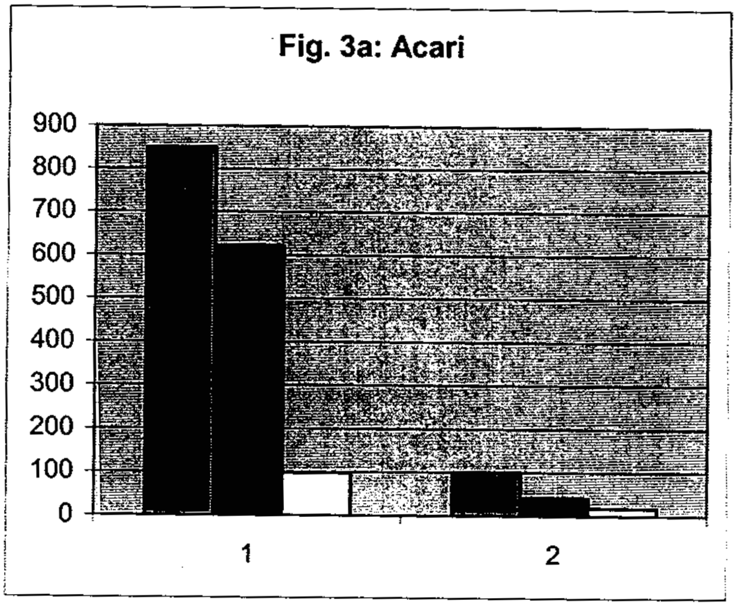


Fig.2. Microarthropods sampled in three experimental plots "treated" as follows: intercropped maize [blue], artificial straw [purple] and mono-maize [yellow]. (field 2D, MPFS; 22/6/2000); all three treatment includes the addition of nitrogen. 1. *Acari* 2. *Entomobyoidea 3. Isotomidae 4. Onychuiridae 5. Myriapoda*



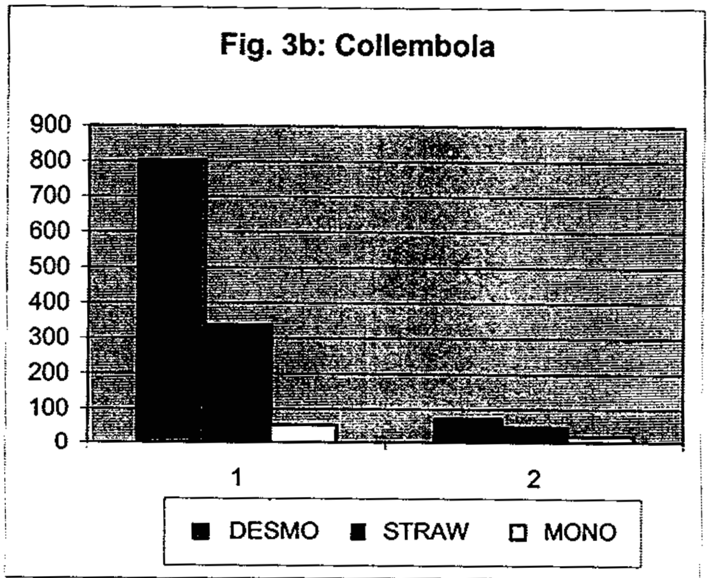


Fig. 3a and 3b. Samples taken in Field plot 2D, but microarthropods found in the litter layer (1) and soil (2) are presented separately; Fig. 3a shows the number of Acari recovered from samples taken in the different treatment plots, and Fig. 3b the Collembola. Note the greater numbers of arthropods recovered from the litter vs. soil.

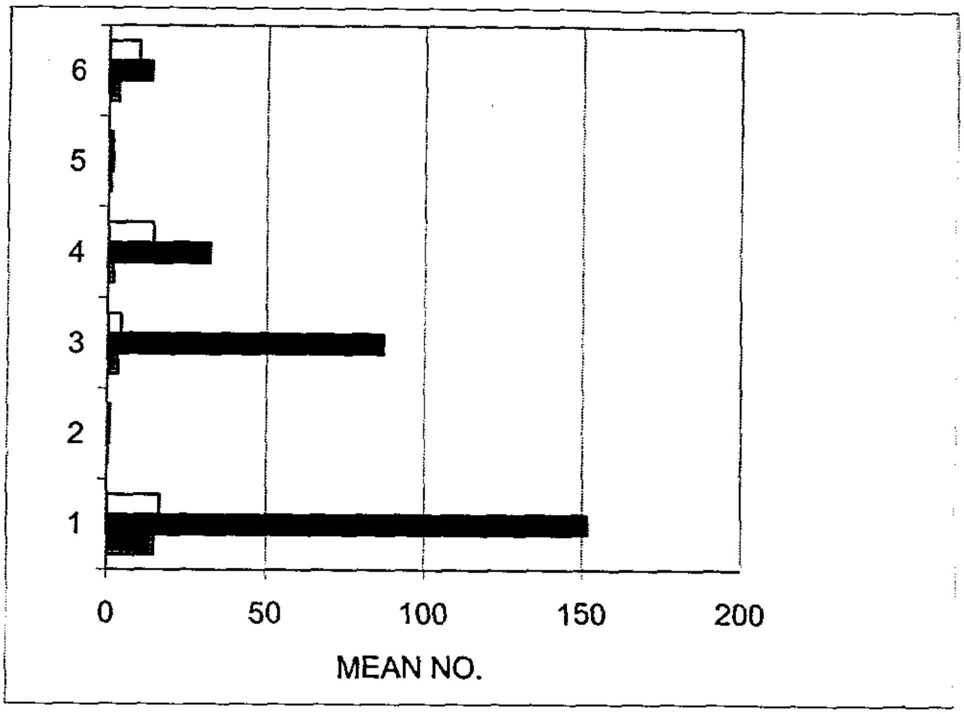


Fig. 4. The vertical distribution of microarthropods in intercropped maize, Field 2D MPFS, 22/6/2000. **Yellow**: Desmodium lower leaves; **Purple:** Litter; **Blue**: Soil. [1] Acari [2] Sminthuroidea [3]Entomobryoidea [4] Isotomidae [5] Myriapoda [6] small Endopterygota

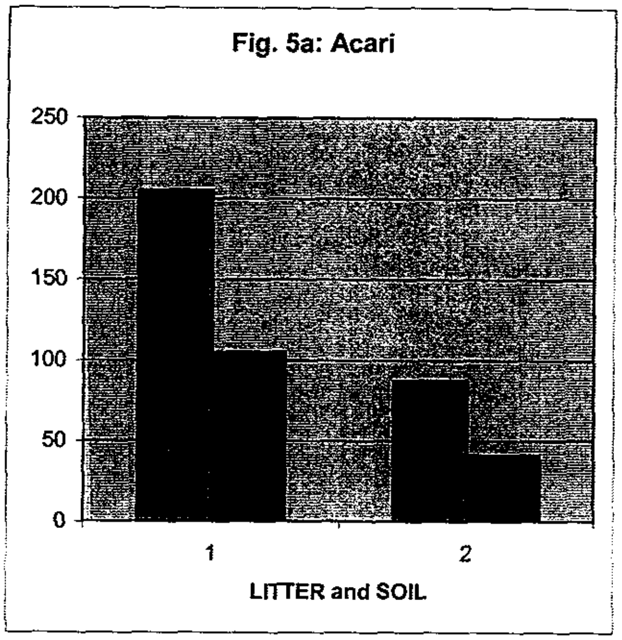
diversity of arthropods in the intercropped plots was significantly higher than in the straw-only plots, suggesting that intercropping creates a multi-dimensional habitat that provides a greater diversity of niches which in turn promotes greater arthropod diversity.

In Figs 3 & 4. the vertical distribution of the animals in the soil profile is presented. As expected, most of the fauna were concentrated in the litter horizon, compared to soil (Fig. 3), and in the lower leaves of the *Desmodium* (Fig.4). Note the scale of the figures, demonstrating the mean number of specimens collected in this sampling (more than 800 for both Acari and Collembola). Such high numbers were probably recovered because the litter was collected by a small trowel rather than a standard core sampler. Litter, which included fragments of the long maize leaves, is difficult to sample with a soil corer. The active role of the arthropods sampled is first of all in litter diminution. They also feed directly on fungi growing in the litter and humus, playing a role in the ecological balance of these microbes. Such activity in annual field crops is limited to the rainy season when soils are moist. The diversity of the arthropods recovered from the mineral soil was very poor. We expected to find Sminthurideae above/at the soil level, or on the lower leaves of the crop, as this is typically where these Collembola are found (see also Fig. 8 below). Small numbers of this group were only found at this interface, with a mean value reaching only 0.8 specimens/sample, a value that is too small to be displayed in Figure 4 due to the scale. It may be that in the present sampling, its occurrence was underestimated as it is usually collected by sweep-netting. Massive populations of this group of Collembola occasionally develop and they can become pests of the crop itself. This phenomenon has largely been documented in leguminous crops (to which *Desmodium* belongs) and outbreaks of sminthuriids have occurred in alfalfa and clover fields in California and Australia {"the alfalfa flea hoppers"} (Rath 1991).

In Fig. 5 the mean number of arthropods collected in random samples taken on three local farms in the Sindo-Lambwe Valley area is shown [each data point is a mean value from 6 samples taken on each farm]. All three farms (Families Ogondi, Ouso and Odek) were "commercial" fields in which maize intercropping was practiced by the farmers [blue] in areas adjacent to traditional maize monocultures [purple]. Soil samples were taken during the first half of August 2000. Species diversity and abundance was particularly rich in samples collected from the intercropped fields, especially with regard to the Collembola (Fig. 5b).

Keeping in mind that maize is a seasonal crop - watered in western Kenya by rain only - we should expect seasonal changes in arthropod density from the rainy- to dry-season. As the intercropping program is based on the assumption that at harvest, the Desmodium will be cut to provide fodder, we would anticipate parallel seasonal changes in arthropod populations; indeed, this was the case (Fig. 6a, b). From a theoretical point of view, it may be desirable to leave a thin litter layer in the field if it resulted in the conservation of higher numbers of microarthropods, and more productive soils. The relatively higher proportion of arthropods recovered from the soil vs. the litter above, may be a reflection of the active downward movement of the Collembola in the soil profile towards moister, cooler layers in response to rising soil surface temperatures and declining surface moisture levels, coupled with the beginning of an aestivation phase.

Microarthropods collected from samples taken from natural habitats in Western Kenya are given for comparison in Figs. 7a and b. The samples were collected at the same time, and using the



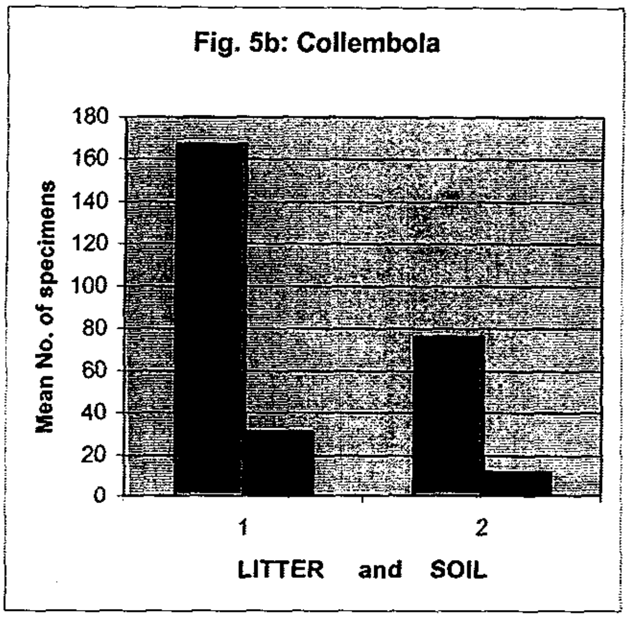
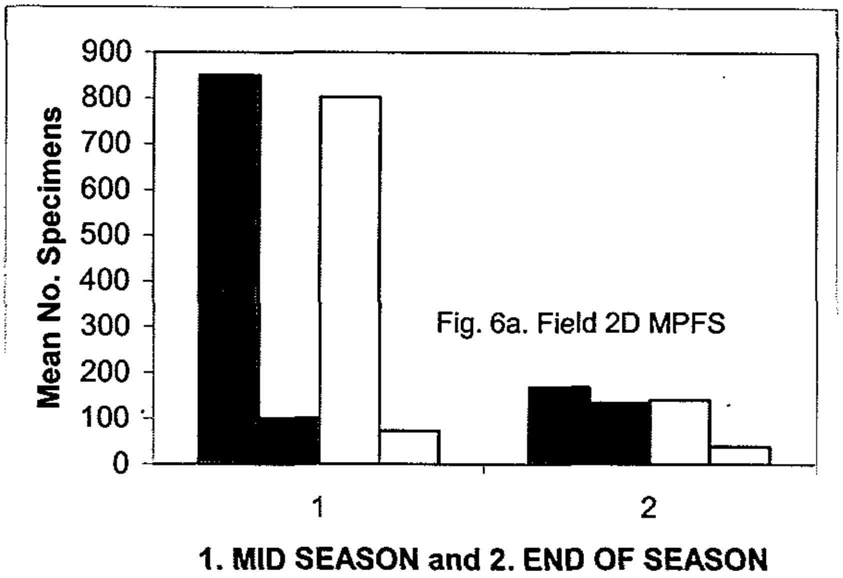


Fig 5a and b. Soil mocroarthropods recovered from samples collected from farmer's fields in the Lambwe Valley during August 2000. In each farm, samples were collected from a maize-Desmodium intercrop field (Blue), and an adjacent mono-maize field (Purple). The data presented are the mean number of specimens/6 samples from the three farms for the Acari (5a) and Collembola (5b).



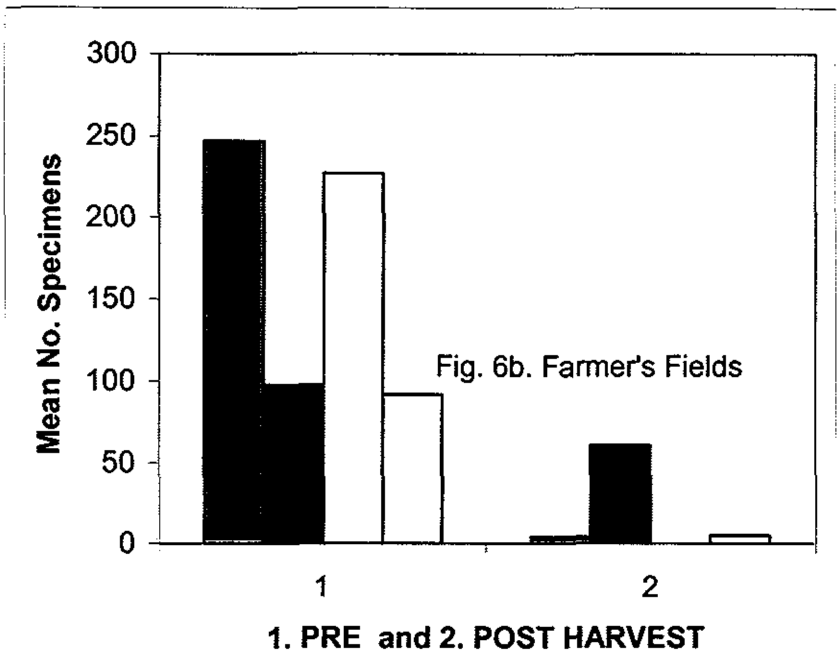


Fig. 6a. Soil microarthropods in Field 2D, as sampled in June 30, 2000 [1], and July 20 [2], respectively. Blue and yellow, represent Acari and Collembola in litter, respectively; purple and green, in soil.

Fig. 6b. The Farm of Mrs. Achola Ogondi; soil microarthropods as sampled in August 1, [1] (pre harvest) and August 24, [2] (post harvest). Blue and yellow, Acari and Collembola in litter, respectively; purple and green, in soil

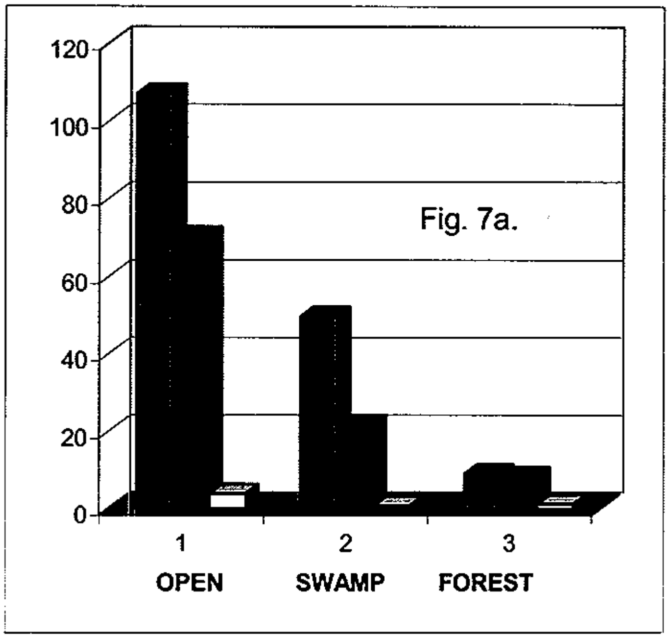


Figure 7. Mean number of arthropod specimens per soil core sample [both litter and soil] in *three* distinct natural habitats in Western Kenya: OPEN FIELD [1] near Kaplong; Forest on the shore of SAIWA SWAMP [2]; and KAKAMEGA FOREST [3].
Fig. 7a. Collembola [Blue] Acari [Purple] and Myriapods [Yellow], sampled June 18, 2000.

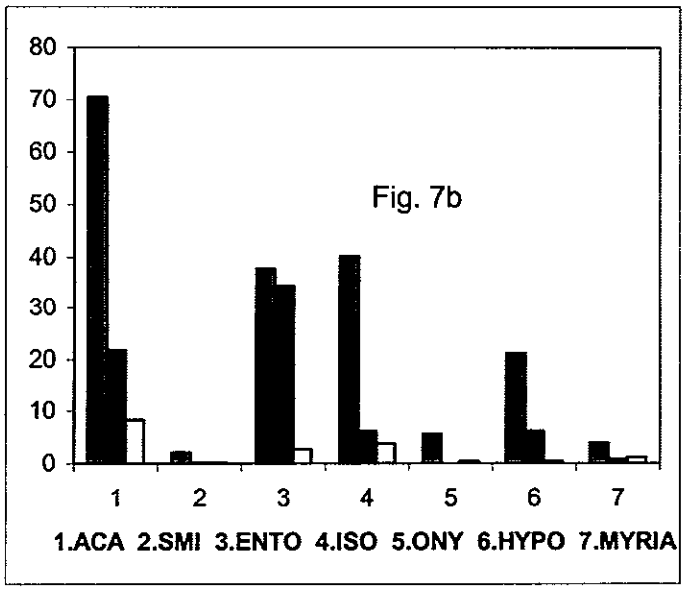


Fig. 7b. Arthropods from the three habitats separated into the following taxa: [1] order Acari; and [2-6] the Collembola families/super families - [2] Sminthuroidea, [3] Entomobryoidea [4] Isotomidae [5] Onychiuroidea [6] Hypogastruridae; and [7] Class Myriapoda. In the figure in each taxa from the 3 localities are shown together (**Blue** = open field; **Purple** = under trees on the shore of Saiwa Swamp; **Yellow** = Kakamega Forest).

same methods, as those taken in maize fields and sheds a broader light on this unique set of observations.

Fig. 7 presents the arthropod fauna recovered from soil and litter samples taken using a standard soil corer in the following 3 habitats: 1) Open field, relatively wet habitat in the Kaplong-Sotic area; 2) Saiwa Swamp (under the trees in the forest surrounding the swamp but not in the swamp itself); 3) Kakamega Forest, under mature trees around "Mama Motero". It is very interesting that the fauna recovered from the natural open field sample was the most diverse, and that the fully grown rain forest climax of Kakamega harbored such a poor range of soil microarthropods, in spite of the thick litter layer and high humidity.

A schematic vertical distribution of Collembolan families as revealed in our studies for the intercropped maize field is presented in Fig 8. In the upper layers, at the base of the *Desmodium* plant cover, we find the Sminthuridea which are completely epigeic. Below, at the litter horizon, are the Entomobryidae, which live in between the litter fragments. Further down, are the Isotomidae. Active in a deeper level of humus and mineral soil are both the Onychiuriids and Hypogastruriids. This is an environment where jumping activity is not possible; indeed, their jumping furca is vestigial. Our data suggest that the build-up these two assemblages (Onychiuridea and Hypogastruridae) in the maize system is negligible. This may be explained by the seasonality or discontinuity of this ecosystem in contrast to the natural habitats sampled (Fig 7b., No. 5 and 6, Blue). However, considerably more research and a search of the literature is required to verify this hypothesis.

3.4. Biodiversity of Kenyan soil organisms: Contribution to the body of knowledge on arthropod biodiversity

"Arthropods form the most diverse group of life. Of the estimated 5-8 million species on earth, probably 3m-6m are insects. Integrated Pest Management (IPM) is a significant practical application of biodiversity because it requires knowledge of species and their inter-relationships" (ICIPE, Science Press,1995). The reported project contributes to our knowledge of soil arthropod biodiversity in nature and in agricultural systems in East Africa. African soil microarthropods are poorly known and their taxonomic description is a big challenge. The Myriapoda (Class Pauropoda), around 1mm in size, are almost unknown from Africa, excluding Madagascar. Only ca 65 specimens have been collected and reported from the whole continent. We collected 13 more belonging to 3 genera, including one novel species: *Allopauropus kenyanus* n.sp. Scheller, (1999). It was recovered from soil collected under an Acacia tree in the Gembe Hills, near Mbita Point, at an elevation of approximately 1700 m, by Broza and Brownbridge. Besides our pioneering work (See First Annual Report) on the identification and description of some of these soil arthropods, samples from the current collection have also been sent to experts in taxonomy in different countries. So far, two new species have been described from lesser-known groups: the Pauropoda and Protura (Scheller 1999, Szeptycki & Broza *In press*). The predominant microarthropods belong to the Collembola and many are presently being identified to species by experts in Europe. A partial list of species identified so far is presented in Table 1.

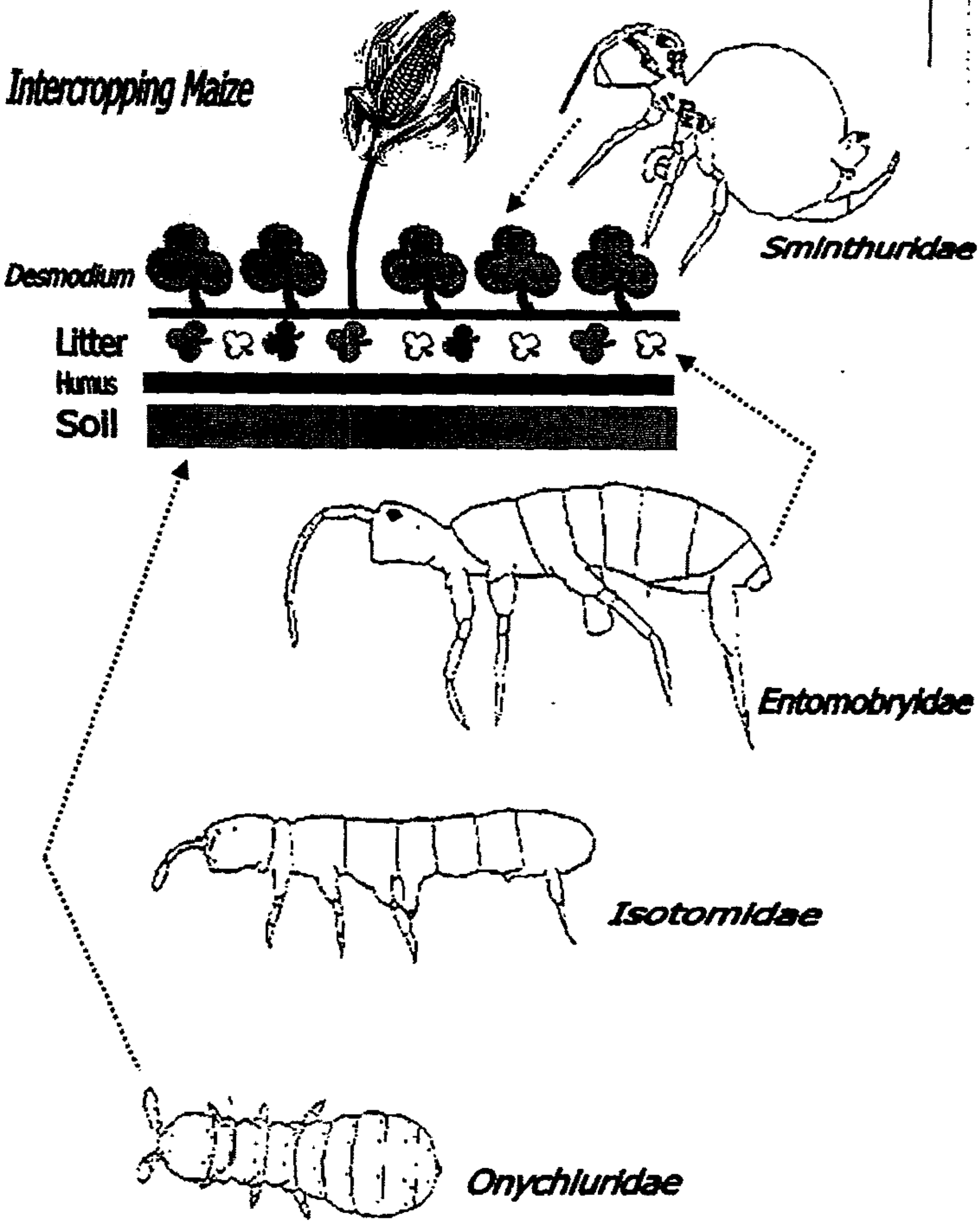


FIGURE 8. The vertical distribution of collembolan families (schematic representation). The two families found lower in the soil profile [Onychuiridae & Hypogstruridae] were almost absent in the seasonal maize field sampled by us [Figs. 1 & 2] compared to the natural open-field habitat [Fig. 7b, Blue].

Table 1. List of Collembola isolated and identified from Western Kenya

Sminthuroidea (Symphypleona)
Sminthurus sp (?)
Hypogastruroidea (Hypogastruridae, Isotogastruridae Neanuridae)
Hypogastrura manubrialis (Tullb, 1869)
Hypogastrura tullbergi Schaeffer, 1900
Afrodontella septembata (Salmon, 1954)
Pseudachorutes niloticus Wahlgren, 1906
Friesea sp.
Odontella sp.
Onychiuroidea (Onychiuridae, Tullbergiidae)
Tullbergia sp.
Isotomidae
Folsomides parvulus Stach, 1922
Cryptopygus thermophilus Axelson, 1900
Isotomiella sp.
Entomobryiidea
Tomocerus sp.
Heteromorus sp.
Entomobrya sp
Lepidocyrtus sp
Pseudosinella sp.
Cyphoderus sp
Dicranocentrus meruensis Wahlgren, 1908

Conclusions

1. The richness of soil micro-arthropod diversity and their overall density in intercropped maize plots are indicative of "healthy" soils and a fertile ecosystem in general. Soil microarthropods have, in intercropped, maize a more suitable [and better] habitat in which to propagate, and contribute to soil health.

2. We may assume that such activity could lead to higher levels of decomposed nutritious elements in such fields, although such parameters were not investigated in the current study.

3. The constraints of modern monoculture agricultural systems, particularly in a seasonal field crop, were clearly shown in comparison to the superior levels of biodiversity observed in the "push-pull" intercropped fields. The resulting benefits to soil health, and ultimately crop yields, are in addition to the direct effects of this practice on *Striga* parasitism and crop protection issues.

4. We showed that soil microarthropods may serve as good bio-indicators, and provide a means of monitoring the environmental impact of agricultural practices; as such, their incidence (abundance and diversity) may be used in future evaluations of new procedures in sustainable agriculture.

5. Although an armyworm outbreak did not occur in the area while we were studying effects of these new cropping practices on biodiversity, we speculate that the push-pull system, which includes a Napier grass perimeter around the plots, would also provide some protection from invasion by AAW.

6. This system was tested by Dr Khan with 600 cooperating farmers in Western Kenya in 2000, and more than 1000 farmers in 2001.

7. This project has made an important contribution to the body of knowledge on arthropod biodiversity in the tropics. The study will continue, using specimens that have been preserved in the collection at the University of Haifa.

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SECTION IV

NON-TARGET IMPACT ASSESSMENT OF AAW CONTROL PRODUCTS: LABORATORY TESTING OF *Bacillus thuringiensis* var. *aizawai* AND AQUEOUS NEEM SEED EXTRACT

4.1. Introduction

As we seek to incorporate sustainable and environmentally benign methods of pest management into AAW control programs, it is commonly assumed that, simply because these pesticides are biologically-derived, they are inherently safer than their synthetic counterparts. When insect pathogens are used, temporarily at least, the microorganisms are present in the environment at significantly higher concentrations than would ever occur naturally. What impact do such high levels of inoculum have on non-target organisms? Similarly, effects of botanical insecticides such as neem on the environment are also unknown. Clearly, there are great benefits to using these materials in place of broad-spectrum chemical pesticides, but there are also implications to such large-scale introductions and we need to evaluate their ecological impact to ensure minimal adverse effects on biodiversity.

In contrast to studies on economically-important insects and natural enemies, very little work has been done to assess effects of microbial and botanical pesticides on non-target beneficials in the soil. All crop treatments, crop residues, etc., ultimately end up in the soil, so it is important to determine the relative risks they pose to the maintenance of a robust soil biota. Large numbers of microarthropods are present in healthy and productive soils; inbalances created by pollutants, agrochemicals or destructive farming practices can irreversibly affect the density and diversity of microarthropod populations, with resulting adverse effects on soil productivity.

Collembola, springtails, are among the most common soil microarthropods. Stable, abundant communities are generally present in well-managed agricultural soils, and they are recognized as key indicator species of soil fertility and health. They play a major role in the decomposition of organic matter, mineralization of soil nutrients, control and distribution of soil microflora; and are critically involved in the breakdown and recycling of crop residues. They also play a vital role in soil aeration. Changes in species diversity and richness provide a means of monitoring changes in their environment. So, there are clear justifications for using Collembola as a representative bioindicator species, against which we can evaluate effects of biopesticides and transgenic crops.

4.2. Materials and Methods

Diet assays. Effects of Bt and the neem-based product Neemix® on *Alophorura hortensis* have been previously presented. These studies highlighted several key areas which warranted further investigation, as well as a need to refine some of the experimental techniques used. Laboratory trials were undertaken at the Univ. of Vermont using *Folsomia candida*. These Collembola were exposed to a commercial formulation of Bt *aizawai* (Xentari®; registered as Florbac in Africa) and neem seed extract via contaminated diet, under controlled conditions. Effects on survival, longevity and fecundity were monitored; in this way, we were able to observe direct toxic effects as well as more chronic effects which can impact long-term survival and population growth.

Figure 1. Collembola assay container showing yeast-agar block.

For the Collembola assays, suspensions of Xentari were prepared in sterile distilled water (SDW) and incorporated into a molten yeast-agar diet cooled to 50 C. After mixing, the diet was cooled to 4 C and held at this temperature until use. For the check treatment, SDW only was added to the molten yeast agar diet. Xentari was added to the diet to achieve the following test concentrations: 2X, X and 0.5X, where 'X' is the recommended field use concentration. The insecticidal activity of the test materials was confirmed by parallel bioassays against *Spodoptera exigua* (beet armyworm), whereby the same test concentrations were incorporated into an artificial insect diet mix and presented to neonate (<12h-old) armyworm larvae. The neem seed extract was produced by crushing and soaking neem seeds in SDW for 20 h and filtering the extract for incorporation into the yeast-agar diet; 20 g of seeds plus 80 ml of water provided a 'standard' 20% stock. This was diluted so that in-diet concentrations of 2%, 1% and 0.5% (v/v) were obtained for presentation to the Collembola. Diluted extract was also tested against beet armyworm to confirm the insecticidal activity of the test concentrations. Fresh diet was prepared weekly, and was refrigerated when not in use.

Blocks of diet were presented to the Collembola in small plastic assay cups (Fig. 1). These contain a thin layer of plaster of Paris plus activated charcoal, which is moistened throughout the experiment, providing an ideal environment for collembolan survival and reproduction. The diet blocks were replaced every 2-3 days (3x/week) over the experimental period. Ten mature *Folsomia* were placed in each cup, and 4 replicates set up; the experiment was replicated twice, providing replication within and over time. The Collembola were presented with treated diet exclusively for 4 weeks, after which survivors were fed for a further 4 wk on yeast-agar only. The number of surviving adults in each assay container was recorded weekly and any cadavers were removed at the same time. Eggs were also removed and counted every 7-d.

Soil assays. These tests were set up for neem seed extract only, as this material also has contact action. The 20% neem seed extract was prepared as described above. Three test concentrations – 2%, 1% and 0.5% (v/v) – were prepared from the concentrate and mixed with sterile soil (3.5 ml/10 g soil; 35% by weight). Sterile distilled water was used as the control treatment. The soil was dispensed in plastic assay containers lined with plaster of Paris, to a depth of approx. 2 cm; holes had previously been made in the base of each cup using a hot needle, prior to the addition of the plaster of Paris. Five 6-week old *F. candida* adults were added to each cup; four containers were set up for each treatment. A small quantity of dried, ground bakers yeast was added to each container as food every 7-d. The soil was kept moist by bottom-watering every 7-d, standing the containers in a reservoir of distilled water, which was absorbed by the plaster of Paris and then moistened the soil. The plaster of Paris prevented the Collembola escaping and prevented water-logging in the soil, while keeping the soil damp. After 8 weeks, the number of Collembola in each container was determined by emptying the soil into a beaker containing 100 ml of distilled water. The Collembola floated to the surface of the water and a digital image was taken, allowing the number of individuals to be counted at a later date.

4.3. Results

When presented to beet armyworm at 0.5 – 2 times the recommended field application rate, all Xentari concentrations caused 100% mortality after 8-d; mortality in the neem seed extract treatments was >80% after 8-d (Table 1). When administered to *F. candida*, however, there was no significant effect on longevity (Fig. 2) or egg production (Fig. 3). In contrast, significantly fewer Collembola survived the neem treatments than the untreated control, and decreased survival in all neem treatments was clearly evident by week 4; by week 6, <20% survived; <5% by week 8 (Fig. 4). Effects on egg production were particularly striking, and significantly fewer eggs were produced by neem-fed individuals from week 2 on (Fig. 5), and essentially ceased altogether by week 3. Effects

Treatment	Mean % Mortality	
	day 3	day 8
Control	0	0
Neem seed extract 0.5%	7.5	82.5
Neem seed extract 1.0%	0	87.5
Neem seed extract 2.0%	2.5	92.5
Control	0	0
Xentari 0.5X	85	100
Xentari 1.0X	90	100
Xentari 2.0X	100	100

Table 1. Mean per-cent mortality of neonate *Spodoptera exigua* 3 and 8-d after treatment with neem seed extract or Xentari (Bt *aizawai*). NSE concentrations expressed as a v/v concentration of extract/ml insect diet; Xentari concentrations: 'X' = recommended field use concentration.

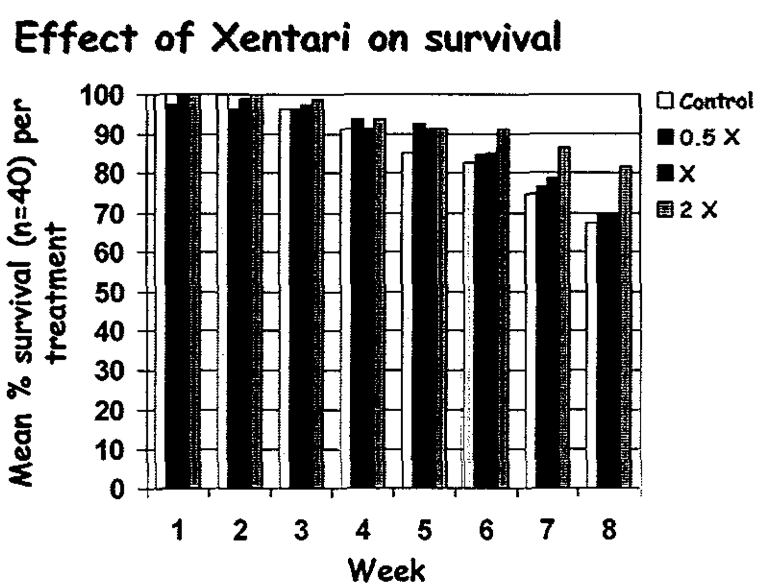

Figure 2. Effect of Xentari on survival of *Folsomia candida* at three test concentrations.

were sustained even when the insects were fed on a yeast-agar only diet (week 4-on).

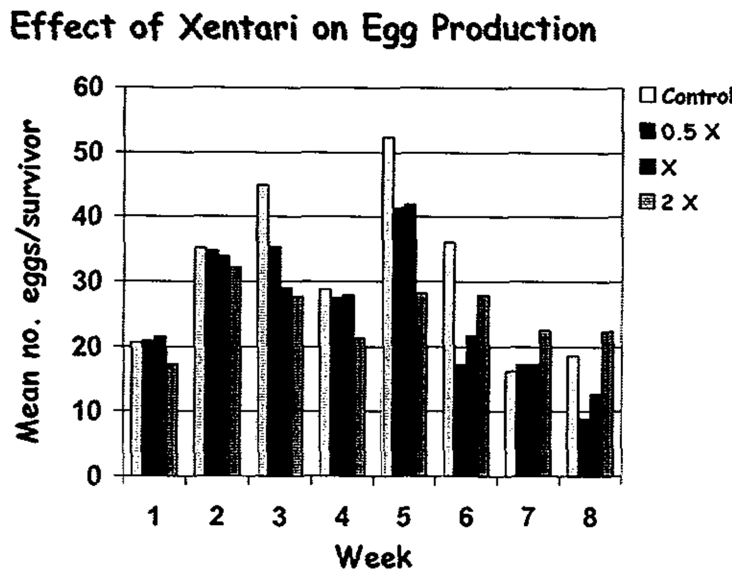

Figure 3. Effect of Xentari on egg production by *F. candida*.

When incorporated into soil, neem seed extract had a significant effect on collembolan population development for all test concentrations (Table 2).

4.4. Discussion

The results suggest that we have little to worry about with applications of Bt (Xentari), given the lack of observed side-effects at the high assay concentrations used. However, there may be some negative side-effects following an application of neem.

Neem is known to have numerous effects on insects, including feeding deterrence, growth regulation and disturbance of reproductive activity. Collembola were observed on the neem-treated blocks and there was clear evidence of feeding activity, although food intake could have been reduced. All of the factors, though, could have contributed to the observed results. Collembola only lay eggs during alternate inter-molt periods; if molting is impaired, this could have resulted in the observed decline in egg production, combined with disruption of ovarian activity. Furthermore, the slow decline in survivorship indicates a chronic impact of the material on collembolan physiology, which affected egg laying and was sustained even when individuals were allowed to feed on non-treated diet after 4-wk.

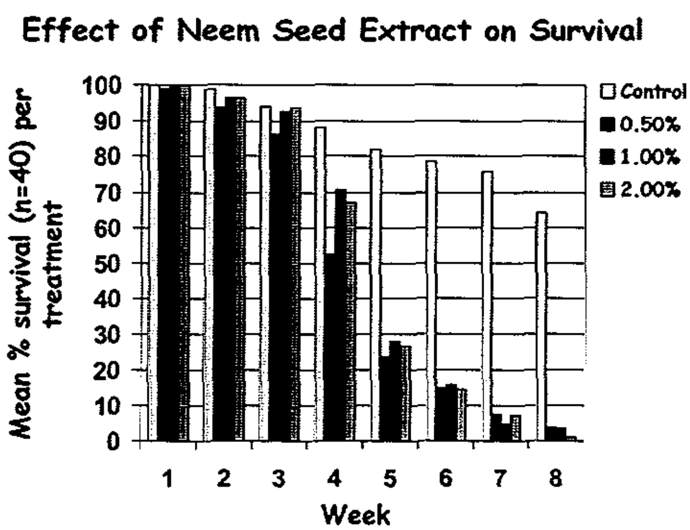

Figure 4. Effect of aqueous neem seed extract on survival of *F. candida*.

While the data suggest that there may be some adverse effects on the microarthropod communities following an application of neem, the results cannot be directly extrapolated to predict what would happen under field conditions. In the trials, the Collembola constantly fed on contaminated diet for an extended period, or were confined to soil contaminated with high concentrations of neem. In a field crop it is unlikely that such high concentrations would reach

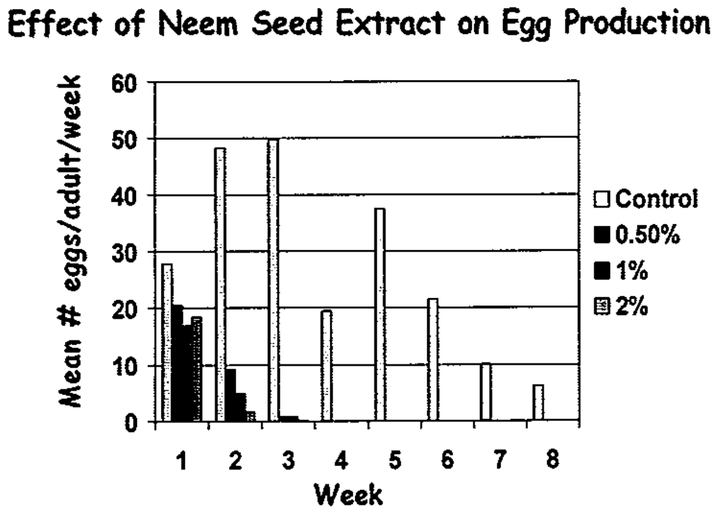

Figure 5. Effect of aqueous neem seed extract on egg production in *F. candida.*

Collembola in their food, or in the soil, nor would plant treatments be made with such regularity so that they would be constantly exposed to such concentrations over an extended period. Furthermore, the lab trials do not take into account the fact that these materials break down in the environment. What they do provide us with, however, is an indication of potential risks that now need to be evaluated under conditions more closely resembling those experienced in the field.

Both of the materials tested are certainly a safer and more acceptable choice than the insecticides currently used against AAW in East Africa. Ultimately, findings must be used to guide decisions on selection and use of pest management tools for AAW in light of the relative risks posed by these materials compared to those posed by synthetic pesticides, and the benefits in terms of increased food production, that would be gained from their use. Furthermore, neem seed extract can be indigenously produced in the areas where it is needed at extremely low cost, and in a timely and responsive fashion. Thus, the rural farming community could have ready access to these control agents. Bt can be produced in-country and neem trees thrive in parts of Kenya, representing an inexpensive and renewable pest management resource.

Treatment	Mean no. Collembola/assay cup
Control	367.3±124.3
NSE 0.5%	8.5±6.6
NSE 1.0%	3.8±2.5
NSE 2.0%	4.3±1.5

Table 2. Mean number of Collembola (adults and immatures) per soil assay container; NSE = neem seed extract; % concentrations given are for the treatment applied to the soil, 3.5 ml per 10 g sterile soil.

APPENDIX

Szeptycki, A. and Broza, M. (2003) *Eosentomon rachelae* n. sp., a new species from Kenya (*Protura, Eosentomidae*) (Manuscript accepted for publication in *Genus*)

NB. Text only. Figures not included.

Eosentomon rachelae n. sp., a new species from Kenya (*Protura, Eosentomidae*)

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Abstract. *Eosentomon rachelae* n. sp. of the "*validum* complex" is described from Kenya. It is the most similar to *E. burahacabanicum* Yin & Dallai, 1985 and to *E. validum* Condé, 1961.

Key words: entomology, taxonomy, *Protura*, new species, *Eosentomon*, Kenya

Introduction

Little is known about the proturan fauna of Sub-Saharan Africa. Actually, from the whole subcontinent (excluding Madagascar and surrounding islands) only 30 species are recorded (Tuxen 1977; 1979; Yin &Dallai 1985). From the Eastern Africa 13 species are known – 5 from Uganda (Condé 1961), 1 from Rwanda (Nosek 1976), 4 from Somali (Yin &Dallai 1985), and 3 from Kenya (Condé 1948)

The present collection was carried out in Western Kenya near the north east tip of Lake Victoria. The lake is at an altitude of 1130 m asl. Its eastern shore is covered by dry savannah, interrupted by a range of volcanic hills built up by lava flows associated with the faulting from which the Syrian-African Rift Valley –just east of this region- evolved. The Gembe Hills are among those very steep volcanic mountains. One of the litter samples (ca. one third of a liter in volume), which was collected while climbing up the hill, yielded more than 30 specimens of the genus Eosentomon.

Eosentomon rachelae n. sp.
(Figs 1-24)

Diagnosis

Eosentomon rachelae n. sp. belongs to the "*validum* complex" of Tuxen (1979). The species of this complex are characterized by the presence of seta *P4a* on urotergites II and III, the presence of labral seta, the lack of anterior setae on urosternite VII, the absence of foretarsal sensilla *b'1* and *c'*, the short empodial appendage of II and III legs, the presence of 4 setae on urosternites IX and X, and more or less reduced setae on urotergite XI.

The complex actually contains 6 African species: *Eosentomon adami* Condé, 1961, *E. angolae* Tuxen, 1977, *E. burahacabanicum* Yin & Dallai, 1985, *E. gabonense* Tuxen, 1978, *E. subglabrum* Condé, 1961, and *E. validum* Condé, 1961 (Tuxen 1964, 1977, 1979, Yin & Dallai 1985).

In the shape of female squama genitalis (with distinct "head" and "beak"), not reduced chaetotaxy on urotergite X, and the position of foretarsal sensillum *t1* (distinctly nearer to *α3* than to *α3'*), the new species is most similar to *burahacabanicum* and *validum*. It differs in the abdominal chaetotaxy and in the length of foretarsal sensilla *t2* and *b'2*. The anterior row of setae on urotergites III – VII contains in *burahacabanicum* 10, 10, 8, 8, 2 setae, in *validum* 8, 8, 4(8), 4, 4 setae, in the new species 10, 10, 6(8), 6, 4(6) setae. Sensilla *t2* and *b'2* in *burahacabanicum* and *validum* are relatively long (about ¾ length of sensillum *a'*) whereas in the new species they are short (about half length of sensillum *a'*).

Description.

Head setae relatively short, subposterior seta 1.6-2 x length of posterior seta. Anterior additional seta, posterior additional seta, seta *m4* and anterior sensillum present.

Pseudoculus small, ovate, with distinct inner line, PR 11-16. Clypeal apodeme strong. Rostral seta alate, subequal to the subrostral. Labrum with truncate apices and deep, narrow notch. Labral seta present. Mandibles with three distinct apical teeth. Digits of galea well developed; median and inner equal and shorter but thicker than outer one. Sensilla of maxillary palp short, lateral shorter than dorsal.

Setae on nota slightly diversified. *P1a* situated posteriorly to line of *P1-P2*, *P2* 1.6-2 x length of *P1*. Length ratio of *P1* : *P1a* : *P2* on mesonotum as 0.8-1 : 1 : 1.3-1.4. *P2a* subequal to *P3a*; *P3a* medially to line *P3 – P4*, setiform. Base of *P4a* close to *P5*, but not connected with it. Tracheal camerae short, slightly dilated basally.

Foretarsus with no sensilla *b'1* and *c'*. Sensillum *a* nearly as long as *c*; *c* short, reaching base of $\gamma 3$; *b* shorter than *a'*; *d* short, reaching base of *z*; *e* and *g* long, with spatulate dilation about half of sensillum length; *f1* spatulate, about 3/4 length of sensillum *e*; *t1* situated nearer to $\alpha 3$ than to $\alpha 3'$; *t3* long, passing base of $\delta 6$; *a'* long, much longer than *t2*, exceeding base of $\alpha 4$, situated slightly distally to level of $\alpha 3$; *t2* and *b'2* short, sub equal and filiform; seta $\delta 4'$ slightly proximal to level of $\delta 4$. BS 1.1-1.2, TR 5.4-5.8, EU 0.8-1.0.

Empodial appendage of II and III leg short; basal seta of III leg (seta *D2*) of normal shape.

Chaetotaxy formula of abdomen:

I	II-III	IV	V-VI	VII	VIII	IX-X	XI	XII
4	10	10	6	4-6	6			
---	----	----	----	----	---	8	8	9
12	16	16	16	16	9			
4	6	6	6	6	0			
---	---	---	---	---	---	4	8	12
4	4	10	10	10	7			

Chaetotaxy formula of urotergite I: 3, 1, 2. Urotergite V and VI with no *A1* and *A3* (on V *A1* sometimes present), VII with no *A1*, *A2* and *A3* (sometimes *A2* present). Seta *P1a* on urotergite I-VI long, on urotergite VII short, situated near *P2*, anterior to it. *P2a* on urotergite II-VI long, situated in half way between *P2-P3*, on urotergite VII as on preceding ones. Seta *P4a* on urotergite II and III present; on urotergite IV-VII setiform. *P1a'* on urotergite VIII with no basal dilation, situated anterior to level of *P2*. Dorsal setae on urotergite X thin, on XI strongly reduced (sometimes hardly visible). Seta *1* on urosternite X subequal to seta *2*.

Antecostae strong with a distinct central lobe. Laterostigma III-IV large with no inner structure. Lateral sclerotisation of urosternite VIII absent. Dorsal lobe of telson has two central pores.

Female squama genitalis short, “head” ovoid with small “beak”. Penis with short basiperiphallar setae.

Measurements (in µm) - imago: Head 118-138, pseudoculus 8-11, subposterior head seta 11-12, posterior head seta 6-7, mesonotal seta *P1* 12.5-16.5, *P1a* 15-17, *P2* 20-24, foretarsus 87.5-96, claw 15.5-17, empodial appendage 13-16.5, maximum body length of expanded specimen about 1200.

Chaetal variability, imagines (11 specimens). Urotergite V: symmetrical (1 s-n) and asymmetrical (1 s-n) presence of *A1*; urotergite VI: asymmetrical (1 s-n) presence of *A1*; urotergite VII: symmetrical (3 s-ns) and asymmetrical (4 s-ns) presence of *A2*.

Holotype: female (collection number 6061): **Kenya**, Gembe Hills (ca 1300 m asl.), 5 km east of the north-east shore of Lake Victoria and in front of Mbita Point, under Acacia tree within thick litter layer. Collection was on 11/04/1998 while the habitat was wet and covered with green grass. leg. M. Broza and M. Brownbridge.
Paratypes: 5 females, 6 males, together with the holotype.
Holotype and paratypes nr 6056, 6060, and 6062-6064 in the collection of the Institute of Systematics and Evolution of Animals of the Polish Academy of Sciences, Kraków, Poland; paratypes nr 6057-6059 and 6065-6067 in The Israeli National Collection, Tel Aviv University.

Name derivation: the new species is dedicated to Rachel (Rahel) Broza.

ACKNOWLEDGEMENT
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[Explanations of figures]

1-13. *Eosentomon rachelae* n. sp.: 1 - head (paratype nr 6062); 2 - anterior part of head, dorsal view (holotype); 3 - distal part of palpus maxillaries (paratype nr 6062); 4 - pseudoculus (paratype nr 6062); 5 - rostral seta (lateral view) (paratype nr 6062) (magnification as fig. 4); 6 - mandible (holotype) (magnification as fig. 3); 7 - galea (holotype) (magnification as fig.3); 8 - pro-, meso- and metanotum (paratype nr 6059); 9 - tracheal camerae (paratype nr 6059); 10 - leg III, anterior view (paratype nr 6062); 11 - leg III, posterior view (paratype nr 6062); 12 - seta *P1a* on urotergite VII (holotype); 13 - seta *P1a'* on urotergite VIII (holotype). Scale: 20 μm

14-18. *Eosentomon rachelae* n. sp.: 14 - foretarsus, exterior view (paratype nr 6067); 15 - foretarsus, interior view (paratype nr 6067); 16 - distal part of foretarsus, exterior view (paratype nr 6067); 17 - distal part of foretarsus, interior view (paratype nr 6067); 18 - foretarsus, dorsal view (holotype). Scale: 20 μm

19-24. *Eosentomon rachelae* n. sp.: 19 - urotergite VI - VIII (holotype); 20 - urotergite IX-XII (holotype); 21 - urosternite IX-XII (holotype) (magnification as fig. 20); 22 - hind margin of urotergite XI (holotype); 23 - antecostae II, III and VII (paratype nr 6065); 24 – female squama genitalis (holotype) (magnification as fig. 22). Scale: 20 μm